THE MORPHOLOGY AND PHYSIOLOGY OF THE SALAMANDER THYROID GLAND.

III. THE RELATION OF THE NUMBER OF FOLLICLES TO DEVELOPMENT AND GROWTH OF THE THYROID IN *Ambystoma maculatum*.

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Owing to the profound changes which the follicular pattern of the human thyroid gland undergoes in various pathological states, the follicular composition of the thyroid has been given careful attention by many students of the thyroid gland.

Martin Heidenhain was one of the first to examine the thyroids of various mammalian species with reference to the follicular pattern. He made the noteworthy discovery that profound differences exist between the different species of this group of animals, regarding the development and morphology of the follicular design of the thyroid. According to Heidenhain two types of follicular design must be distinguished, an association type to which belong the thyroids of the dog and cat, and a dissociation type to which belong the thyroids of the cattle and of man. In the association type the follicles which develop from the primary cell columns remain permanently in epithelial continuity, while in the dissociation type they become separated. In both types secondary "fusion" may take place. Thus in the thyroids of dog and cat secondary communication (canalization) between the adjacent follicles of an epithelial column may take place, if the quantity of secretion increases, and lead to the formation of tubuli possessing a continuous lumen. The same process may, at times, occur in man, at the stage of the primary cell columns, before splitting up into separate follicles has taken place; if it does occur, it acts inhibiting upon the

[1] Part of the work reported in this article was carried out with the aid of the "Julius Friedenwald Fund for Medical Research" of the University of Maryland.

process of splitting up. Heidenhain made some observations also on the growth and multiplication of follicles. He states that real budding of follicles is very rare; normally each cell of the primary cell columns is destined to develop sooner or later into one individual follicle. No new follicles, beyond the original number of cells, develop. In cattle, however, budding may be observed during the period of most rapid growth, just after birth. What appear to be buds in man, are only young follicles developing out of cells which failed to separate at the time when the other cells separated, the result of an incomplete dissociation. As to a multiplication of follicles Heidenhain observed that even in case of real buds these never become separated; likewise a cleavage of larger into smaller follicles does not occur.

In 1923 Williamson and Pearse (14, 15) published a study of the follicular pattern of the human thyroid gland and reported conditions essentially different from previous observations. According to these investigators the human thyroid is composed of gland-units a certain number of which make up one thyroid lobule. These units consist of an endothelial capsule the lumen of which is part of the general lymphatic system and communicates with other units and with the general lymphatic system, by means of a stalk-like lymph capillary. Very oddly in this lymphatic capsule are contained blood vessels, the capillaries of the gland-unit, which enter the capsule through the stalk-like hilus. In addition to the blood vessels each capsule contains probably one, possibly several coiled and freely floating epithelial columns. These epithelial columns of each gland unit, being the real glandular elements of the thyroid, are completely independent of the columns of other gland units, except for the lymphatic and blood-vascular communications. The solid epithelial columns represent the primary resting condition of the gland-unit. When functioning either of two completely different states—and only one of them at a time—may develop from these solid cell-columns. In both cases a lumen appears in the center of the cell column; in one case it becomes filled with colloid, in the other case with a more fluid substance. The colloid is not a secretion; the secretion is represented by the more liquid substance which, it is asserted, leaves the lumen of the epithelial tube by way of "intracellular" tubules. Normally units in each of these three states are found together in one thyroid. But a unit which elaborates colloid cannot, at the same time, elaborate secretion. Before this is possible, it must revert to the original condition of the primary solid cell column.

So far these assertions, together with many others to be found in the publications referred to and adhered to by Williamson and Pearse in their more recent articles (16, 17) are mere statements; to support them the authors have furnished a surprisingly small number of facts. For reasons outlined specifically in a previous article we cannot subscribe to these theories at large; some of them are rather incoherent and others are based upon grossly wrong observations and interpretations. But in connection with the present article we are mostly interested to know whether in the human thyroid what impressed most observers as separate vesicles are in reality only parts of continuous tubules. It is well known and has been pointed out by Heidenhain and others that the human thyroid, in certain states, does seem to be composed of numbers of coiled, continuous tubules rather than of isolated vesicles.

Upon inspection of the thyroid of an exophthalmic goiter gland [1] we have convinced ourselves that within the larger lobules smaller areas can be distinguished, which are separated from the vicinity by finer connective-tissue strands; the follicles located within these areas suggest frequently that they might be merely parts of one larger, continuous tubule and sometimes resemble in a striking manner the condition found in *Ambystoma opacum*, illustrated in Fig. 39, Plate I. of a previous paper (11); as will be mentioned presently, in this species there really exists a condition somewhat similar to the one described by Williamson and Pearse.

Since 1922 the senior author of this article has been engaged in a study of the thyroid gland of the salamander *Ambystoma opacum;* the results of this work have been published in a number of previous articles (2 to 13). In this species of salamander the thyroid, during development, may follow either of the two types distinguished by Heidenhain (1) in mammals. But whichever

[1] This gland was received from Dr. A. S. Blumgarten, of the German Hospital in New York; it gives me pleasure to thank Doctor Blumgarten for his courtesy.

type it follows, the final result is, in the main, the formation of a large and coiled tube, distended with secretion to a varying degree in different places of its course, and resembling the condition described by Williamson and Pearse for the thyroid of man. The whole gland of the marble salamander may be compared, at that stage, with one single unit of the human thyroid gland. This continuous tube forms through a process of fusion of the smaller follicles, very much as described by Heidenhain, except that in case of previous dissociation really separated follicles fuse with each other. It was found that the development of this tube-like follicle, together with the elaboration and storage of the secretion product (a mixture of unstainable and, mainly, stainable colloid) coincides with the larval period. It represents, what was called, the developmental or storage phase of a functional cycle the second phase of which is the "functional phase" or phase of colloid release, which coincides with metamorphosis and is characterized, as regards the follicular pattern, by a complete collapse of the follicular tube, caused by the sudden release of the secretion product from the lumen of the follicles. The functional phase is followed again by the storage phase which now is initiated by an enormously increased elaboration of predominantly unstainable colloid. Like in man and other mammals—and unlike the assertions made by Williams and Pearse—the two secretion products, the chromophobe and the chromophile colloid, are always elaborated in and excreted from the same cells at the same time and into the same secretion-space, although in a varying proportion.

The important feature in the thyroid of the marble salamander and the one of especial interest in connection with the present article is the existence of a phase during which the small primary follicles fuse with each other to form a large tube, sometimes resembling a coiled tube, sometimes exhibiting a more bag-like appearance, which is distended with the secretion product of the cells. At the time when these observations were made the thyroid gland of *Ambystoma tigrinum* and of the axolotl, in addition to the thyroid of the marble salamander, were studied on sections and it was noticed that the follicular patterns of these glands differ markedly from that of the thyroid of the marble salamander; the presence of a particularly large follicle was not noticed in these glands.

It was thought desirable to make a comparative study of the follicular pattern of several different species of salamanders, to see what was essential in the follicular pattern of the thyroid in general and whether differences in the follicular pattern corresponded to certain specific physiological differences of the thyroid apparatus. As at the same time large numbers of thyroids of experimental material had to be examined, the need was felt of developing a method which would enable us to make accurate examinations of the follicular pattern without the laborious work of sectioning and as soon as possible after the removal of the organs from the animals. It was found that upon maceration with 40 per cent. nitric acid the thyroid can be taken apart completely into its individual follicles, the size and shape of the follicles can be studied and the exact number of follicles can be ascertained for each individual thyroid gland. A brief account of the results thus obtained was published in the *Anatomical Record* (Uhlenhuth, 12).

In the present article the results obtained with this method for the thyroid of the salamander *Ambystoma maculatum* only will be reported; the thyroids of other species of salamanders have been successfully investigated with this method and publication of these investigations will be made soon. It should be mentioned here that the same method can be applied very successfully also to the thyroids of warm-blooded animals (dog) and of man.

Method.

The animals were anesthetized with chloretone (10 cc. of an 0.5 per cent. aqueous solution of chloretone plus 40 cc. of a 30 per cent. Ringer solution) and pinned up in a tray under 30 per cent. Ringer solution. The thyroids were removed in the usual way and placed at first into a dish containing a 30 per cent. Ringer solution. From this solution they were placed directly into a 40 per cent. nitric acid (Merk's Blue Label). It is best to leave them in the acid for from 12 to 24 hours and then to transfer them into a large dish filled with tap water. The water should be changed at least once. The glands treated in this fashion should be dissected as soon as possible to avoid softening of the epithelial walls of the follicles. If this procedure is employed the connective tissue will be found disintegrated sufficiently to give way to the slightest pulling, while the follicles show great resistance and can be separated from one another without the least injury to the epithelial walls. The colloid assumes a yellowish tint, the cells are whitish. In case a part of the epithelial wall is scraped off or components belonging to one single follicle are torn asunder, the defect of the epithelium and the smooth, yellowish surface of the denuded colloid can be noticed at once.

Each thyroid was completely dissected into its individual follicles and the absolute number of follicles was ascertained (Table I., column 6). Representative follicles as to shape and size were then selected and their outlines drawn at a magnification of $\times$ 33 (Zeiss Binocular Dissection Microscope, Oc. 2, Obj. a_2).

In order to determine the relative number of the follicles, *i.e.* the number of follicles per unit of thyroid volume, it would have been necessary to determine the volume of each thyroid gland. As only in a few cases the thyroid volume was ascertained another, less accurate method was resorted to. Of each thyroid the largest optical section was drawn with the aid of a camera lucida, at a magnification of $\times$ 33, after the thyroids had been removed from the acid into the water. With the aid of a planimeter the area in cm^2. of each outline drawing was determined (Table I., column 8). By dividing the number of follicles over the area the number of follicles per cm^2. area was found for each thyroid (Table I., column 7). This method would give correct results only if the cross section of each thyroid were a circle. As some thyroids are less cylindrical than others, the values for the relative numbers of follicles as recorded here are only approximately correct. No conclusions, however, were drawn from these values beyond those suggested by the continuity and persistency of the change of this value with the progressing increase in the size of the thyroids and age of the animals.

Material.

The investigations reported in the present article were carried out on the species *Ambystoma maculatum*, in spring and summer

1925 and 1926. With the exception of the larvæ of the series CCLXII. and the sex-mature animals of the series CCLXX. the animals were reared from eggs in the laboratory. All animals were kept under the same conditions except where mention is made of the contrary. All animals of the same series are the offspring of the same female, except the animals of series CCLXII. and CCLXX. The entire material was collected by Mr. George Gray in the vicinity of Woods Hole, Mass.

The Number of Follicles.

1. Average number of follicles: The average number of follicles of the thyroid gland of the spotted salamander, as calculated from the thyroids of 88 normal animals examined since 1925, is 39.7 follicles. There is, however, a considerable variability from the average; the smallest number of the follicles in one thyroid was 5 follicles, the largest number 72 follicles (Table I., column 6).

Explanation to Table I.

The early larval stages (from No. 1 to No. 29) are arranged according to body length in mm.; the older larval stages and the metamorphosed animals are arranged according to stage, as during these periods of life the stage and not the size of the animal determines the condition of the thyroid gland.

The areas of the thyroids of the first seven animals (column 8) were drawn from the fresh gland, in all the other glands the areas were drawn after removal of the thyroids from the nitric acid into water. Comparison of the area before and after the acid treatment showed a shrinkage of the thyroid, which decreased the area by approximately 18 per cent. of the area of the untreated thyroid; therefore 18 per cent. should be subtracted from the values of the area of the first seven animals, to obtain the correct measurement.

Abbreviations.

E.pr.sl. . . . . Eyes protrude slightly.
E.pr.d. . . . . Eyes protrude distinctly.
E.pr.c. . . . . Eyes protrude considerably.
Yell.N. . . . . The even coloration of the earlier larval stages has been replaced by a yellow network on dark background.
Op.sh. . . . . Opercular pouch, on ventral side, has started to grow to the body wall and has become shallow as compared to the previous larval stage.
G. . . . . Gill slits.
Pieces 1 day. . . . . Cast off, for the first time, some small pieces of epithelium, 1 day before examination of thyroid.
Sh.Sk. . . . . Shed his skin completely.
L. . . . . Placed on land (moist filter paper).
Eats. . . . . Started taking food again.

Table I.

Normal *Ambystoma maculatum*, Examined Since 1925.

Number.	Series.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm². Area.	Area, in cm². of Largest Optical Section of Thyroid at a Magnification of × 33.
1	CCLXXI. 3	2.5 toes; 1 balancer left.	7.5	10	—	—	0.16
2	CCLXXI. 1	2.5 toes; no balancer left.	8.0	10	—	—	0.16
3	CCLXXI. 7	3.0 toes; no balancer left.	8.4	12	—	—	0.12
4	CCLXXI. 9	3.5 toes; hind limb buds out.	9.5	21	—	—	0.33
5	CCLXXI. 10	[1] 3.5 toes; hind limb buds out.	9.6	21	—	—	0.25
6	CCLXXI. 11	4.0 toes; hind limb buds split in 2.	9.6	28	—	—	0.41
7	CCLXXI. 12	6.0 toes.	11.1	29	—	—	0.14
8	CCXLVII. 32	9.0 toes.	13.4	126	*28*	80	0.35
9	CCLXXI. 14	8.5 toes.	15.1	44	*38* [2]	42	0.9
10	CCXLVII. 33	9.0 toes.	16.4	126	*28*	62	0.45
11	CCLIV. 10	Eyes protrude slightly.	16.5	118	*33*	—	—
12	CCXLVII. 28	9.0 toes.	17.6	74	*32*	22	1.45
13	CCXLVII. 34	Larval.	17.9	126	*32*	35	0.95
14	CCLXXIV. 1	Larval.	20.0	65	*52*	43	1.2
15	CCLXXIV. 2	Larval.	21.3	66	*28*	22	1.3
16	CCLXXI. 16	Larval.	21.4	60	*34*	16	2.2
17	CCLXXI. 19	Larval.	21.5	63	*58*	25	2.3
18	CCLXXI. 20	Larval.	22.2	63	*24*	13	1.9
19	CCLXXI. 17	Larval.	22.8	62	*31*	13	2.5
20	CCLXXI. 18	Larval.	22.8	63	*38*	16	2.4
21	CCLXXX. 1	Eyes protrude slightly.	23.4	65	*50*	14	3.6
22	CCLXXX.*a* 5	Eyes protrude slightly.	23.8	76	*42*	26	1.65
23	CCLXXX.*a* 2	Eyes protrude slightly.	24.0	70	*52*	22	2.35
24	CCLXXI.*a* 1	Eyes protrude slightly.	25.7	75	*42*	17	2.55

[1] For changes of the color pattern before and after metamorphosis see Uhlenhuth, J. Experim. Zoöl., 1917, XXIV., 237–301 (p. 243).

[2] The number of follicles could not be ascertained as this thyroid consisted of a solid and continuous mass of epithelial cells, in which only four follicles were differentiated.

TABLE I. (*continued*).

Number.	Series.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm². Area.	Area, in cm². of Largest Optical Section of Thyroid at a Magnification of × 33.
25	CCLXXI.*a* 2	E.pr.sl.;[1] larval color pattern.	27.9	77	*51*	16	3.1
26	CCLV. 1	E.pr.sl.	28.3	91	*37*	13	2.8
27	CCLXXIV.*a* 1	E.pr.distinctly; larval color patt.	29.2	92	*49*	14	3.4
28	CCLV. 2	E.protrude.	30.2	91	*25*	7	3.6
29	CCLXXIV.*a* 2	E.pr.distinctly; larval color patt.	32.3	92	*29*	7	4.2
30	CCLXXI.*b* 2	E.pr.sl.; yellow network.	27.2	79	*41*	14	2.9
31	CCLXXI.*a* 4	E.pr.d.; yellow network.	31.1	77	*35*	14	3.4
32	CCLXXI.*b* 3	E.pr.d.; yell.N.; Operculum shallow.	30.6	87	*56*	17	3.35
33	CCLXXI.*b* 5	E.pr.d.; yell.N.; Op. shall.	31.1	88	*72*	11	6.85
34	CCLXXIV.*a* 3	Pieces 1 day; E.pr.d.; yell.N.	33.7	96	*32*	11	2.85
35	CCLXXI.*a* 5	Pieces 1 day; E.pr.cons.; yell.N.; O.sh.	35.9	88	*35*	13	2.75
36	CCLXII. 6	Yellow N.	36.0	125	*10*	3	3.5
37	CCLXII. 4	Late larval; Eyes protrude.	37.3	125	*35*	7	4.7
38	CCLXII. 10	Late larval; Eyes protrude.	37.4	127	*30*	7	4.6
39	CCLXXI.*a* 6	E.pr.consid.; yell. spots; Op. and G. closed.	35.6	89	*65*	12	5.4
40	CCLXII. 8	Begins Sh.Sk.; Op.sh.; G. open.	37.3	126	*37*	8	4.65
41	CCLXII. 7	Begins Sh.Sk.; Op. very sh.; G. open.	42.0	126	*25*	3	7.45
42	CCLXXI.*b* 6	Just shedding; O. and G. partly open.	32.4	91	*47*	14	3.45
43	CCLXXI.*a* 3	Just shedding; Op. and G. closed.	27.8	77	*30*	10	2.95
44	CCLXII. 2	Just shed Sk.; Op. closed; G. partly op.	42.4	126	*31*	4	8.2
45	CCLXXI.*a* 12	Just shed Sk.; Op. and G. partly open.	—	91	*28*	9	3.05
46	CCLXXI.*b* 1	Just shed Sk.; Op. and G. closed.	27.1	77	*43*	17	2.5
47	CCLXII. 5	Sh.Sk. ½ hour; Op. and G. nearly closed.	40.1	125	*5*	1	4.6
48	CCLXII. 9	Sh.Sk. 3 hours.	37.0	126	*44*	8	3.5
49	CCLXXIV. 3	Sh.Sk. 0 days.	22.5	68	*48*	19	2.5

TABLE I. (*continued*).

Number.	Series.		Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm². Area.	Area, in cm². of Largest Optical Section of Thyroid at a Magnification of × 33.
50	CCLVII.	1	Sh.Sk. 0 days; Op. closed; G. open.	33.0	95	*63*	16	4.0
51	CCLXXI.*a*	14	Sh.Sk. 0 days; Op. and G. nearly closed.	35.2	95	*24*	8	3.2
52	CCLIX.	6	Land 0 days; Op. and G. closed.	30.7	120	*33*	11	3.0
53	CCLXXIV.*a*	5	Sh.Sk. and L. 1 day; G. closed; Op. very sh.	—	106	*26*	10	2.5
54	CCXXXVI.	9	Sh.Sk. and L. 1 day; Op. and G. closed.	29.9	118	2		2.4
55	CCLVI.	1	Sh.Sk. 1 day; Op. closed; G. open.	35.6	91	*52*	10	5.5
56	CCLVII.	5	Sh.Sk. and L. 1 day; Op. and G. closed.	27.8	123	*33*	10	3.35
57	CCLXII.	11	Sh.Sk. and L. 1 day; Op. and G. closed.	36.1	128	*30*	7	4.3
58	CCLXII.	12	Sh.Sk. and L. 1 day; Op. and G. closed.	32.4	128	*37*	10	4.65
59	CCLXXI.*a*	7	Sh.Sk. and L. 1 day; Op. and G. closed.	33.8	89	*50*	12	4.2
60	CCLXII.	1	Sh.Sk. 2 days; L. 1 day; Op. and G. closed.	40.9	120	*39*	5	7.2
61	CCLXXI.*a*	9	Sh.Sk. and L. 2 days; Op. and G. closed.	31.6	89	*52*	11	4.6
62	CCLXXI.*b*	7	Sh.Sk. and L. 2 days; Op. and G. closed.	31.7	92	*71*	16	4.5
63	CCLXXI.*a*	8	Sh.Sk. and L. 2 days; Op. and G. closed.	33.5	89	*42*	8	5.2
64	CCLXXI.*a*	13	Sh.Sk. and L. 2 days; Op. and G. closed.	34.8	92	*44*	6	7.9
65	CCLXII.	13	Sh.Sk. 3 days; L. 2 days; Op. and G. closed.	34.8	130	—	—	6.2
66	CCLXXI.*b*	8	Sh.Sk. and L. 3 days; Op. and G. closed.	28.6	95	*67*	21	3.2
67	CCLXXI.*a*	10	Sh.Sk. and L. 3 days; Op. and G. closed.	32.2	89	*57*	13	4.25
68	CCLXII.	3	Sh.Sk. 4 days; L. 3 days; Op. and G. closed.	39.0	124	*54*	—	—
69	CCLXII.	14	Sh.Sk. 4 days; L. 3 days; Op. and G. closed.	41.4	130	*36*	4	8.15
70	CCLXXI.*b*	4	Sh.Sk. and L. 4 days; G. absorbed.	28.8	87	*32*	17	2.35
71	CCLXXI.*a*	11	Sh.Sk. and L. 4 days; G. absorbed.	29.6	89	*43*	10	4.45
72	CCLXXIV.*a*	4	Sh.Sk. and L. 5 days; Eats 1 day.	35.4	100	*47*	16	2.95
73	CCLXXI.*a*	15	Sh.Sk. and L. 6 days.	33.1	96	*42*	7	5.7
74	CCLXXI.*a*	18	Sh.Sk. and L. 11 days.	32.2	100	*63*	15	4.2

TABLE I. (*continued*).

Number.	Series.		Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm^2. Area.	Area, in cm^2. of Largest Optical Section of Thyroid at a Magnification of $\times$ 33.
75	CCLXXI.*a*	19	Sh Sk. and L. 11 days. Eats 1 day.	33.1	101	*36*	15	2.45
76	CCLXXI.*a*	20	Sh.Sk. and L. 12 days. Eats 4 days.	34.9	101	*50*	9	5.4
77	CCLXXI.*a*	16	Sh.Sk. and L. 13 days.	31.1	96	*36*	12	3.05
78	CCLXXI.*a*	17	Sh.Sk. and L. 13 days.	31.6	96	*44*	10	4.45
79	CCLXXI.*a*	21	Sh.Sk. and L. 13 days.	35.2	101	*26*	6	4.5
80	CCLXXI.*a*	22	Sh.Sk. and L. 17 days. Eats 8 days.	35.3	102	*47*	14	3.6
81	CCLXXI.*a*	23	Sh.Sk. and L. 20 days.	31.8	104	*72*	25	2.9
82	CCLXXI.*a*	24	Sh.Sk. and L. 189 days; killed February.	40.5	279	*39*	10	4.1
82*a*	CCLXXI.*a*	25	Sh.Sk. and L. 197 days; killed April.	46.0	328	*36*	10	3.5
83	CCXXXIV.	12	Sh.Sk. 379 days; killed August.	51.5	474	*23*	7	6.6
84	CCXXXIV.	4	L. 403 days; killed August.	49.0	476	*24*	6	4.15
85	CCXXXIV.	13	Sh.Sk. and L. 539 days; killed February.	53.5	649	*11*	2	4.96
86	CCXXXVI.	10	L. 532 days; killed February.	42.5	650	*34*	7	4.9
87	CCXXXVI.	12	Sh.Sk. and L. 532 days; killed February.	54.2	657	*26*	8	3.3
87*a*	CCXXXIX.	10	L. 591 days; killed April.	67.0	703	*40*	3	11.2
88	CCLXX.	1	Adult female; killed May 1926.	103.5	—	*34*	3	11.2
89	CCLXX.	2	Adult female; killed May 1926.	93.0	—	*53*	5	11.0
90	CCLXX.	3	Adult female; killed May 1926.	88.0	—	*24*	3	8.6
91	CCLXX.	4	Adult female; killed May 1926.	101.3	—	*30*	2	13.6
92	CCLXX.	5	Adult male; killed August 1926.	86.2	—	*48*	3	15.5
93	CCLXX.	6	Adult male; killed August 1926.	83.5	—	*33*	3	10.8
94	CCLXX.	7	Adult female; killed Febr. 1927.	90.3	—	*52*	5	9.6
95	CCLXX.	8	Adult female; killed March 1927.	84.3	—	*59*	6	9.2
			Average			39.7	13.3	

The difference of the number of follicles is considerable not only between the thyroids of different individuals, but also between the two thyroids of the same individual (Table II.*a*).

Table II.*a*.

Differences in the Number of Follicles between the Two Thyroids of the Same Individual.

Series Number of Animal.	Left Thyroid.		Right Thyroid.	
	Number of Follicles.	Area of Largest Optic Section of Thyroid in cm².	Number of Follicles.	Area of Largest Optic Section of Thyroid in cm².
1. CCLXXI.*a* 23.	72	2.9	50	3.2
2. CCLXXI.*a* 24	39		32	
3. CCXXXIV. 13.	11	5.0	19	6.5
4. CCXXXVI. 10.	34	4.9	37	4.2
5. CCXXXVI. 12	26	3.3	23	2.8

2. Relation of the number of follicles to the size of the gland: The differences in the number of follicles are independent of the differences in the size of the glands. The number of follicles is not determined by the size of the gland. This fact may be ascertained by direct observation. It is also evident from comparison of the number of follicles with the values of the area of the thyroids. One thyroid, for instance (No. 1 in Table II.*a*) measured 2.9 cm²., but contained 72 follicles, while the thyroid of the other side of the same animal contained only 50 follicles, although it measured 3.2 cm².

In Table II.*b* are recorded the number of follicles, the thyroid-volume [1] and the number of follicles per cm³. of thyroid-volume for both thyroids of each of two animals. It will be noticed

[1] The thyroid-volume was calculated in the following manner. The largest and the smallest longitudinal optical sections of each gland were outlined and the areas measured as before. The area of the largest longitudinal optical section was multiplied by the largest diameter of the smallest longitudinal optical section and the area of the smallest longitudinal optical section multiplied by the largest diameter of the largest longitudinal optical section. The two products were added and the sum was divided over 2.

that the left thyroid of animal No. 1 contained fewer follicles than the right one, yet the volume of the left gland was larger than that of the right gland. In animal No. 2 of Table II.*b* the

Table II.*b*.

Differences between the Two Thyroids of the Same Individual in Respect to the Absolute Number of Follicles and to the Number of Follicles per cm³. of Thyroid Volume.

Series Number of Animals.	Left Thyroid.			Right Thyroid.		
	Number of Follicles.	Volume of Thyroid in cm³.	Number of Follicles per cm³. of Thyroid Volume.	Number of Follicles.	Volume of Thyroid in cm³.	Number of Follicles per cm³. of Thyroid Volume.
1. CCXXXIV. 13	11	7.16	1.5	19	11.3	1.8
2. CCXXXVI. 12	26	4.2	6.2	23	3.2	7.1

larger thyroid contained more follicles, absolutely, than the right one, but relatively the number of follicles was smaller in the left one than in the right one.

The variability of the number of follicles of the thyroids of different animals will become evident from an inspection of Table I. There are a number of thyroids among the smallest ones (less than 3 cm². area), which are composed of over 50 follicles, while among the largest thyroids there is, for instance, one which measures 13.6 cm²., but contains only 30 follicles, and another one measuring 8.6 cm². and containing 24 follicles.

In Table II.*c* the absolute number of follicles, the thyroid-volume and the number of follicles per cm³. of thyroid-volume are recorded. As will be noticed, the variability of the number of follicles, independent of the size of the gland, is not a merely apparent one caused by an inexactness of the method of expressing the size of the thyroid by the area of the largest optical section; a similar variability is noticeable, if the size of the thyroid is expressed in volume.

3. Relation of the number of follicles to the growth of the thyroid: From an inspection of Table I. it will be obvious

that, although the variability of the number of follicles is considerable, it does not show any relation to the growth of the gland. In a general way the number of follicles remains unchanged during the entire life of the animal. This circumstance is born out most clearly by the values for the relative number of follicles (Table I., column 7). The relative number of follicles is at first high (80, 42, 62), decreases rapidly with the size of the gland and in adult animals is found to be between 2 and 5.

Table II.*c*.

Variability of the Thyroid Gland in Respect to the Absolute Number of Follicles and to the Number of Follicles per cm³. of Thyroid Volume.

Series Number of Animal.	Left Thyroid.			Approximate Age of Animal.
	Number of Follicles.	Thyroid Volume in cm³.	Number of Follicles per cm³. of Thyroid Volume.	
1. CCLXXI.*a* 24	39	5.3	7.4	1st year
2. CCLXXI.*a* 25	36	4.7	7.7	
3. CCXXXIV. 13	11	7.2	1.5	2d year
4. CCXXXVI. 10	34	7.9	4.3	
5. CCXXXVI. 12	26	4.2	6.2	
6. CCXXXIX. 10	40	21.7	1.8	3d year
7. CCLXX. 7	52	23.7	2.2	Adult
8. CCLXX. 8	59	21.3	2.8	

The thyroid gland of the spotted salamander does not grow by multiplication of its follicles, but by an increase in the size of its individual follicles.

There is, in this species, no definite relation also between the number of follicles and the development of the gland, as will be discussed presently.

Size and Shape of the Follicles.

1. Types of follicles: The size and shape of the follicles shows an extreme degree of variation; nevertheless a number of general types of follicles can be distinguished.

In a general way it may be stated that the shape of the follicles is the more irregular the larger the follicles are.

(*a*) The smallest follicles (primary follicles) are usually round and in most thyroids strictly spherical (Fig. 1, *a*). But in nearly every thyroid there is one or several large follicles which are fairly round, possessing no diverticula at all (Fig. 1, *b* and *c*).

FIG. 1. Various types of follicles found in the thyroid gland of *Ambystoma maculatum*. Outlines drawn with the aid of a camera lucida, at a magnification of × 33 (Zeiss Binocular Dissecting Microscope, Oc. 2, Obj. a_2).

The cross-lined areas indicate solid follicles.

(*b*) Another kind of follicles with smooth surface are the elongate, tube-like follicles of cylindrical shape (Fig. 1, *d* and *e*).

(*c*) The simplest type of diverticulated follicle is represented

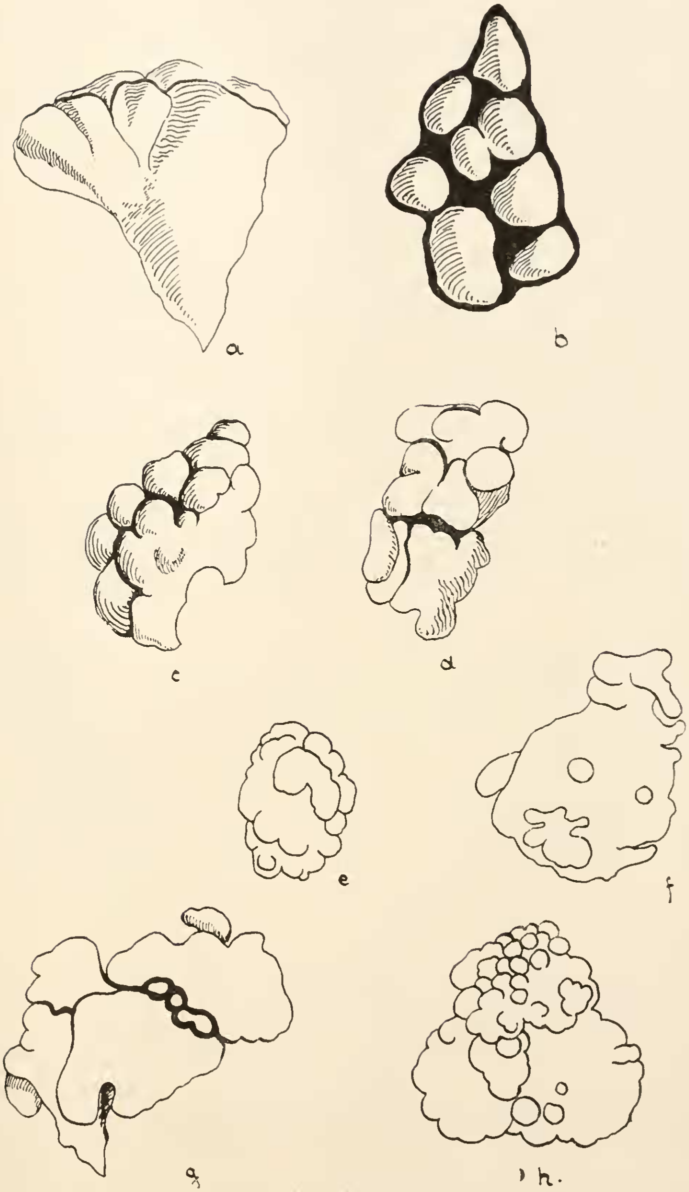


FIG. 2. Various types of follicles, found in the thyroid gland of *Ambystoma maculatum*. Drawn with the aid of a camera lucida, at a magnification of × 90 (Zeiss Binocular Dissecting Microscope, Oc. 4, Obj. *a*3). *a*, side view; *b*, view of the base of the same follicle; *c*, side view; *d*, view of the base of the same follicle. *f*, a pollicle taken from the thyroid of *Notophthalmus torosus* (CCXLVI. 2).

by fairly large, round follicles bearing on their surface one or several bud-like, spherical diverticula of the same size as the spherical primary follicles (Fig. 1, *h* and *i*). This type is rare in the spotted salamander, but is found frequently in the tiger salamander as will be shown in a subsequent paper.

(*d*) A very conspicuous type is represented by follicles of conical or pyramidal shape (Fig. 1, *j*, *k*, *l* and Fig. 2, *a* and *b*). The apex tapers into a point; from the base project two or more finger-like diverticula. In the simplest case these follicles consist of two component follicles communicating at the apex by a small hole and diverging towards the base. But usually the distance along which the component follicles are fused is much more considerable; a conical follicle results, which, at the base, is split into two finger-like processes. In many thyroids, especially noticeably so in the thyroids of sex-mature adults, a dozen or more finger-like diverticula may project from the base, while the apex is still single and pointed (Fig. 1, *k* and Fig. 2, *a* and *b*).

(*e*) Another type of follicles, especially frequent in animals metamorphosed for some time and in adults, is represented by follicles bearing so many diverticula that their shape has become completely irregular (Fig. 1, *m* and *n*, Fig. 2, *c*, *d*, *e* and *f*).[1]

(*f*) While in each of the previous types one major follicle is recognizable, bearing on its surface a varying number of smaller follicles, there is another type, the "composite follicle," which consists of two or more equally large major component follicles (Fig. 1, *o* to *s*, Fig. 2, *g* and *h*). The component follicles may communicate with one another along a considerable area of their circumference (Fig. 1, *o* and *s*, Fig. 2, *g* and *h*), or communication may be established merely by a tiny hole (Fig. 1, *p*, *q* and *r*). Particularly interesting are those follicles whose component follicles are in close apposition with one another along a considerable portion of their entire circumference, yet communicate only by a very small hole (Fig. 1, *p*) or by a short stalk. Each of the component follicles may possess a relatively smooth surface or may bear, in its turn, numerous smaller diverticula

[1] *f* has been taken from the thyroid of *Notophthalmus torosus*, but is representative for many follicles of the spotted salamander.

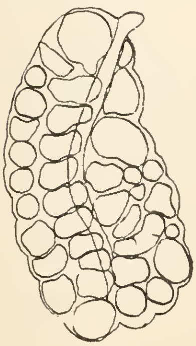
on its surface. In the latter case extreme irregularity and complexity of the follicle results.

(*g*) The smaller follicles of each type may be without a lumen, consisting of a solid mass of cells. Solid follicles are found more often among the spherical primary follicles than among the other types. In the large follicles one or several diverticula may be found solid (Fig. 1, *h*).

(*h*) Not infrequent are flat follicles; they still contain some colloid, but the lumen is very small, slit-like.

2. General arrangement of the follicles: Sometimes no definite design can be found in the arrangement of the follicles. But in most thyroids the follicles exhibit very definite relations.

The follicles are frequently arranged in perfect rows which follow the course of the main vessel (external jugular vein); the original columnar arrangement which the thyroid shows before the differentiation into follicles may be preserved thus in the thyroid of the adult (Fig. 3).



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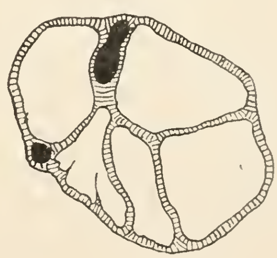

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FIG. 3. Diagram of an entire thyroid gland (Zeiss Binocular Dissecting Microscope, Oc. 2, Obj. a_2; magnified $\times$ 33), showing the arrangement of the follicles in rows parallel to the external jugular vein.

FIG. 4. Diagram of a cross section through a thyroid gland (Zeiss Binocular Dissecting Microscope, Oc. 4, Obj. a_3; magnified $\times$ 90), showing the arrangement of the follicles. White, follicles; cross lined, connective tissue; black, large veins.

The follicles are frequently so arranged, within the cross-section of the organ, that each follicle not only borders, with its central extremity, on one of the large vessels, but also comes to lie, with its peripheral surface, within the periphery of the whole organ (Fig. 4).

At each (caudate and craniate) end of the thyroid is usually one particularly large, fairly round follicle. The small spherical follicles—which in *A. maculatum* are always scarce—are usually located in the periphery of the organ (in *A. tigrinum* some thyroids contain as many as 25 per cent. extremely small follicles; here they are often crowded together into nests surrounded on all sides by larger follicles).

Characteristic is the location of the large, finger-like follicles. The apex of these follicles is always located in the center of the cross-section of the gland (Fig. 4). The finger-like diverticula point towards the periphery and their free ends come to lie, with their entire surface, within the periphery of the gland.

3. Relation of size and shape of follicles to growth and development of the thyroid: While the number of follicles bears no relation to either growth or development of the thyroid, the size and shape of the follicles is distinctly correlated to the size of the gland and, therefore, indirectly to the developmental stage of the gland.

The development of the thyroid of the spotted salamander, during the very early stages, up to the completion of the limb development, is very similar to the development of the thyroid in the marble salamander, as described in a previous paper (11). If the entire organ is removed and examined in fresh condition under the microscope, it will be seen that it consists, at first, of a solid mass of cells, devoid of any orderly arrangement of the cells. Soon this mass of cells assumes a columnar shape. The column is at first a continuous mass of epithelial cells. Gradually it becomes differentiated into small spherical groups of epithelial cells and finally each of these groups develops a lumen in its center, and differentiates into a primary follicle. At that stage maceration in nitric acid was attempted, but was unsuccessful, in as much as separation of the primary follicles was impossible. Microscopical examination shows that the connective tissue has not yet invaded the epithelial anlage; the follicles are not yet separated by connective tissue, but are in perfect epithelial continuity with each other. The youngest animal in which isolation of the follicles was partly successful was animal No. 9 in Table I., a larva possessing nearly the complete number of toes. The right thyroid of this animal was isolated into 38 portions. Of these 29 were recognized to be small individual spherical globules; although, as microscopical examination of the left thyroid showed, these globules contained a distinct lumen, the contents must have been different from the colloid of later stages, as it did not turn yellow in the nitric acid. Eight other portions of the same thyroid were of irregular shape and consisted probably partly of primary follicles and partly of solid cell-groups, all of which were connected directly by epithelial tissue. The last portion was quite large constituting a considerable part of the entire organ and being partly columnar in shape, representing in its entirety a solid mass of epithelial cells (Fig. 5, *A*).

The youngest larvæ in which complete isolation of the follicles is possible measure about 20 mm. body-length (No. 14 in Table I.) and are in the possession of completely developed toes. In the thyroids of these animals only a small number of spherical primary follicles is present (about 10 to 15 per cent.), the other follicles vary in size up to five times the size of a primary follicle and are slightly irregular in outline (Fig. 5, *B*).

By the time metamorphosis approaches (Fig. 5, *C*) the smallest follicles are still of the size of primary follicles, they are almost invariably spherical or at least roundish and their relative number is unchanged (10 to 15 per cent.). The largest follicles have not only increased considerably in size, but have become irregular in shape; among them may be represented all the types enumerated previously. Between these two extreme types of follicles transitional forms are found.

About at the time of the first skin shedding flat follicles containing almost no colloid and corresponding to the collapsed follicles found regularly in *A. opacum* after the first skin shedding are conspicuous; an exact study of that condition has not been possible with the maceration method.

The follicular pattern as found in the late larval stages remains almost unchanged during the entire life of the individual. In the adult animal the largest follicles are still larger and more irregular. Among the smallest follicles there may be found, in the first and second year, still one or several small, spherical

FIG. 5. Four thyroid glands of different developmental stages of *A. maculatum*, showing the changes of the follicles in size and shape. *a*, outlines of the largest optical section of each thyroid gland; below the outlines of representative follicles of each gland. Cross-lined areas indicate a solid cell mass. Outlines drawn with the aid of a camera lucida, at a magnification of × 33 (Zeiss Binocular Microscope, Oc. 2, Obj. a_2).

A. Thyroid of a larval animal (No. 9 in Table I.) of 44 days of age and 15.1 mm. body length.

B. Thyroid of a larval animal (No. 14 in Table I.) of 65 days of age and 20.0 mm. body length.

C. Thyroid of a larval animal (No. 29 in Table I.) of 92 days of age and 32.3 mm. body length, approaching metamorphosis.

D. Thyroid of an adult, sex-mature male (No. 92 in Table I.) of 86.2 mm. body length (age unknown).

or at least roundish follicles of the size of a primary follicle. In the sex-mature adults, however, the smallest follicles are larger than the primary follicles and may be five or more times as large as a primary follicle (Fig. 5, *D*).

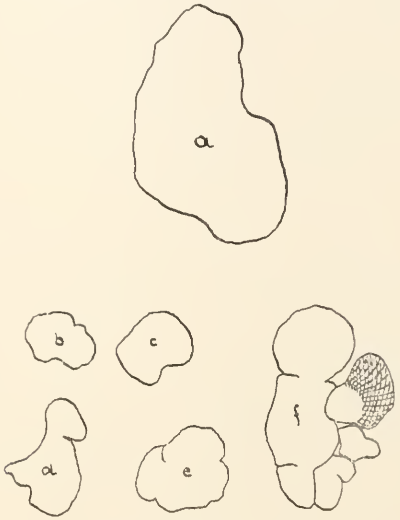

FIG. 6. Thyroid and follicles composing it, of specimen No. 47 in Table I., an animal which was examined one half hour after the first skin shedding. The whole thyroid gland was composed of only five follicles, one especially large and making the largest part of the entire organ.

Outlines drawn with the aid of a camera lucida, at a magnification of $\times 33$ (Zeiss Binocular Dissecting Microscope, Oc. 2, Obj. a_2).

a, outlines of largest optical section of the whole thyroid gland.

b, *c*, *d*, *e* and *f*, outlines of the follicles composing the gland.

THE MODE OF THYROID GROWTH.

As may be calculated from the values recorded in column 8 of Table I. (area in cm^2.) the volume of the adult thyroid gland of *A. maculatum* is about 680 times as large as the volume of the thyroid of a larva at the time at which the first toes are developed.

This considerable growth might take place in two ways, either by an increase in the size of the follicles or by an increase in the number of the follicles.

The diverticulation of the follicles, increasing with the size of the gland, the presence of follicles which consist of several component follicles communicating often only by a narrow stalk and finally the presence of relatively very small follicles in the thyroids of practically every stage, if taken together, would suggest that the follicles develop buds which, after attaining a certain size, separate from the mother follicles. If this assumption were correct, the number of follicles should increase with the size of the gland and more follicles should be found in the adult than in the larval gland. In reality the number of follicles stays unchanged. *The growth of the thyroid is effected entirely by an increase in the size of the follicles.*

Nevertheless it could be imagined that the follicles composing a particular thyroid at a certain stage are not the same follicles composing the same thyroid at another stage. The follicles could divide continuously without increasing in number, if just as many follicles disintegrate as new ones are forming. Although under certain experimental conditions, as will be described in a subsequent article, disintegration of many follicles may be produced, positively no atrophic or degenerating follicles were ever found in the normal thyroid of *A. maculatum.* This observation is fully in accord with the condition found through histological study in the thyroid of *A. opacum.*

Although the shape of the follicles suggests strongly a process of budding and cleavage of larger into smaller follicles, it is obvious that in reality *separation of the bud-like diverticula from the mother follicles and cleavage of larger follicles into smaller follicles does not take place.*

The presence of bud-like diverticula and of composite follicles may be explained in two different ways. The diverticula may form as an outgrowth from the wall of the original follicles or they may be the result of fusion of smaller follicles with larger follicles. Histological study of the thyroid of *A. opacum* showed convincingly that follicular fusion plays a very important rôle in the growth of the thyroid of this animal. Conditions in *A. maculatum* are somewhat less favorable to analyzing this phase of the problem. Several facts, however, suggest, that in the spotted salamander processes of follicular fusion are going on during a large part of the animal's life. As pointed out, small spherical follicles are present in the thyroids of almost every stage. A complete analysis of the problem hinges around the question whether the primary follicles found in any particular stage of an individual thyroid gland are the same as those present at a later stage or whether new primary follicles are developing continuously during the growth of the thyroid. The primary follicles retain practically the same size from an early larval stage up to the winter of the second year of the animal's life. If the primary follicles found during the second winter are the same as those present at the early larval stages, it would mean that they have not been taking part in the general follicular growth. It would be difficult to explain this peculiarity. Neither pressure nor lack of nourishment can be invoked as the cause of it; the primary follicles are located almost invariably at the periphery of the organ and are frequently in close vicinity of large vessels. Moreover the primary follicles do not by any means lack the potential ability of growing; on the contrary they may finally attain a considerable size as may be seen in the thyroids of completely adult, sex-mature animals.

If the primary follicles seen in the thyroid during the later stages are not the same individuals as those seen at the earlier stages, then it would mean that new primary follicles are forming continuously during the growth of the thyroid, but do not increase in number, because a corresponding number fuse with the follicles already present, either while they are still small and spherical (bud-like diverticula) or after they have reached a larger size (composite follicles). Two facts were observed, which would fit in well with this interpretation. As has been mentioned above, follicles are found frequently, which consist of several large component follicles communicating with each other sometimes merely by a small hole. Since cleavage of follicles does positively not occur, this condition may indicate a fusion in its earliest beginning. Particularly suggestive are such follicles which are in closest apposition along a considerable surface, but communicate nevertheless only by a small opening in the adjacent walls (Fig. 1, *p*). The small spherical follicles present very frequently a relation to the large follicles, which is very similar to the relation of the bud-like diverticula and is different from it only by the lack of communication (Fig. 1, *f*).

The other circumstance of interest in this connection is the frequent presence of tiny solid cell masses in thyroids of nearly every stage. These cell masses are difficult to find in the macerated glands and it may be for that reason that they have been overlooked in many glands. With the method of maceration a minute analysis of these cell masses is impossible. It should be pointed out, however, that they are epithelial in nature, distinctly different from the connective tissue as well as from the clusters of red blood corpuscles. It is believed that they correspond to similar cell masses found in histological thyroid sections of *A. opacum* and called "reserve cell masses" in a previous paper (11). Microscopical study of these cell masses in the marble salamander showed that they are the source for continuous development of small young follicles. Most probably the same condition prevails in the spotted salamander; the reserve cell masses split off, during a large part of the animal's life, new primary follicles which in turn fuse with the old follicles. The increase in the size of the smallest follicles as observed in the glands of adult animals indicates that in these glands development of new primary follicles has ceased and no further fusion of the older primary follicles with the large follicles takes place.

These facts suggest that *at least a certain number of diverticula are the result of fusion of smaller follicles with larger ones.*

It was observed that in *A. opacum* intracellular colloid globules may enlarge within the cells of the follicular wall, come to lie free within the epithelial wall and finally fuse with the main mass of intrafollicular colloid (11). While they lie free in the epithelial wall they may, sometimes, form a small, bud-like protrusion on the outside of the follicle. Possibly some of the very smallest diverticula in *A. maculatum* are formed by such intracellular colloid droplets.

It is of interest to note that Heidenhain found separation of buds from the mother follicles and cleavage of large into small follicles entirely absent in the thyroid of mammals. Our findings in the thyroid of *A. maculatum* corroborate entirely Heidenhain's observation in the thyroid of mammals. As mentioned above, Heidenhain distinguishes two types of thyroids, an association and a dissociation type. The thyroid of *Ambystoma maculatum* belongs to the dissociation type. The separation of the follicles, however, is absolutely complete. The bud-like diverticula of the large follicles, if they occur at all, are, according to Heidenhain, primary follicles not yet isolated. In *A. maculatum* the bud-like diverticula cannot be interpreted this way; as indicated by the perfectly smooth and spherical shape of the primary follicles isolation takes place at an early stage.

Fusion of follicles isolated previously was not observed by Heidenhain in the mammalian thyroid gland; it takes place, according to this author, only between the lumina of follicles which have not been separated before. As described in a previous article (11) this kind of fusion is not uncommon in the marble salamander. In the spotted salamander, it seems, fusion is accomplished between follicles which have been really separated before. We are in doubt, however, that in the material and with the method used by Heidenhain a secondary fusion of previously separated follicles could be detected, if it did exist.

Epithelial reserve cell masses from which follicles are split off continuously were not observed by Heidenhain in the mammalian gland. He considers the small follicles of the cattle thyroid as the primary result of the general dissociation taking place at a very early stage. According to his views large numbers of the small primary follicles remain permanently unchanged, from the time of their separation till old age. In *A. punctatum* these primary follicles are absent in the adult glands indicating that finally the primary follicles begin to grow too.

Exceptional Cases of the Follicular Pattern.

In *A. opacum* the follicular pattern shows a very definite relation to metamorphosis. Two types of follicular design are characteristic. As the follicles begin fusing with each other shortly after the completion of the limb-development, the thyroid consists, some time before metamorphosis takes place, for the most part of a large, tube-like follicle distended greatly with colloid. The other characteristic pattern is found just after the first skin shedding. At this time the large, tube-like follicle collapses nearly in its entire extent, as the colloid is released from its lumen, and then forms a nearly solid and continuous mass of cells.

As will be evident from the facts described above neither of these conditions was found to be an integral part of the developmental cycle of the thyroid of *A. maculatum*. Nevertheless we found two thyroids which recall in a most conspicuous manner the structure characteristic for the thyroid of *A. opacum*.

The first case is represented by an animal of series CCLXII., animal No. 47 in Table I.; its thyroid was examined one half hour after the first skin shedding. Only one of the two thyroids was macerated. It consisted of only 5 follicles (Fig. 6). Small spherical follicles were missing entirely. Four of the five follicles were of good size, but did not show anything unusual. The fifth follicle, however, was excessively large, making up the largest portion of the entire gland. It consisted of seven component follicles of rather smooth outlines; one of these was partly solid. On the whole this follicle resembled the large bag-like follicle found normally in the thyroid of *A. opacum* and developing there by fusion of the smaller follicles. In *A. maculatum* this condition was found only once among 120 animals.

The second case is represented by the thyroid of animal No. 54 in Table I., which was killed one day after the first skin shedding. This thyroid consisted almost in its entirety of a solid mass of cells, devoid of any lumen and colloid. In addition 11 smaller masses were isolated. But only 4 of them were follicles of vesicular nature; the other seven were solid cell masses; it was suspected that they had been broken off, in the process of dissecting the macerated gland, from the main mass. This thyroid resembles closely the condition of the thyroid found normally in *A. opacum* shortly after the first skin shedding and called in a previous paper (11) "stage of colloid release." In *A. maculatum* it was found only once among 120 animals. But in another species, *A. jeffersonianum*, of which only few thyroids were examined by the maceration method, exactly the same condition was met with in an animal which was examined one day after it had been placed on land (CCXXXIII. 7). Animals of the same series from which the *A. maculatum* described above had been taken were examined more than one year after the first skin shedding; none of them showed this condition of almost complete continuity of the epithelium and lack of colloid. It is therefore certain that it was not a peculiar thyroid structure characteristic for the offspring of one particular female.

It may be said, then, that although as a rule the follicular pattern of the thyroid of *A. maculatum* does not show any striking changes corresponding to certain functional changes, such changes may develop in rare cases. They resemble the changes taking place regularly in *A. opacum*. It should be noted especially that while the development of one particularly large tube-like follicle is not an integral phase in the functional cycle of the thyroid of the spotted salamander, it does occur occasionally.

On the whole the differences of the follicular pattern of the thyroid, existing between two so closely related species as *A. maculatum* and *A. opacum*, are very striking. Yet the occurrence of the two exceptional cases of follicular pattern in *A. maculatum* shows, that in the thyroid of this species the ability of developing the follicular pattern of *A. opacum* is potentially present.

Comparison of the thyroids of the two species of salamanders examined furnishes, we believe, further evidence to show that the plasticity of the endocrine system is very considerable in the group of amphibians.

Variability of the Number of Follicles.

Owing to the extreme variability of the number of follicles it is difficult to determine whether or not a change in the number of follicles can be effected by any particular experimental procedure. Nevertheless, as we have seen in experiments to be described in a subsequent paper (13), it is possible to change so considerably the number of follicles, that the degree of the change exceeds the degree of variability. In the present article we will discuss only such variations the causes of which are not entirely understood.

As was pointed out, when reference was made to Table I. the number of follicles varies between 5 and 72, if all of the animals examined are included. If, however, animals of the same parentage are compared, the variability is considerably less, as may be seen from an inspection of Table III. All animals

Table III.

Series CCLXXI.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm.² Area.	Area, in cm.² of Largest Optical Section of Thyroid at a Magnification of × 33.
3	2.5 toes; 1 balancer left.	7.5	10	—	—	0.16
1	2.5 toes; no balancer left.	8.0	10	—	—	0.16
7	3.0 toes.	8.4	12	—	—	0.12
9	3.5 toes; hind limb buds.	9.5	21	—	—	0.33
10	3.5 toes; hind limb buds.	9.6	21	—	—	0.25
11	4.0 toes; hind limb buds split in two.	9.6	28	—	—	0.41
12	6.0 toes.	11.1	29	—	—	0.14
14	8.5 toes.	15.1	44	*38*	42	0.9
16	Larval.	21.4	60	*34*	16	2.2
19	Larval.	21.5	63	*58*	25	2.3
20	Larval.	22.2	63	*24*	13	1.9
17	Larval.	22.8	62	*31*	13	2.5
18	Larval.	22.8	63	*38*	16	2.4
a 1	E.pr.sl.	25.7	75	*42*	17	2.55
a 2	E.pr.sl.	27.9	77	*51*	16	3.1
a 4	E.pr.d.;[1] yell.N.	31.1	77	*35*	15	3.4
a 5	Pieces 1 day; yell.N.; Op. very shall.	35.9	88	*35*	13	2.75
a 6	Yellow spots; Op. and G. closed.	35.6	89	*65*	12	5.4

TABLE III. (*continued*).

Series CCLXXI.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm.² Area.	Area, in cm.² of Largest Optical Section of Thyroid at a Magnification of × 33.
a 3	Just shedding Sk.; Op. and G. closed.	27.8	77	*30*	10	2.95
*a*12	Just Sh.Sk.; Op. and G. partly open.	—	91	*28*	9	3.05
*a*14	Sh.Sk. 0 days; Op. and G. nearly closed.	35.2	95	*24*	8	3.2
a 7	Sh.Sk. and L. 1 day; Op. and G. closed.	33.8	89	*50*	12	4.2
a 9	Sh.Sk. and L. 2 days; Op. and G. closed.	31.6	89	*52*	11	4.6
a 8	Sh.Sk. and L. 2 days; Op. and G. closed.	33.5	89	*42*	8	5.2
*a*13	Sh.Sk. and L. 2 days; Op. and G. closed.	34.8	92	*44*	6	7.9
*a*10	Sh.Sk. and L. 3 days; Op. and G. closed.	32.2	89	*57*	13	4.25
*a*11	Sh.Sk. and L. 4 days; Gills absorbed.	29.6	89	*43*	10	4.45
*a*15	Sh.Sk. and L. 6 days.	33.1	96	*42*	7	5.7
*a*18	Sh.Sk. and L. 11 days.	32.2	100	*63*	15	4.2
*a*19	Sh.Sk. and L. 11 days; Eats 1 day.	33.1	101	*36*	15	2.45
*a*20	Sh.Sk. and L. 12 days; Eats 4 days.	34.9	101	*50*	9	5.4
*a*16	Sh.Sk. and L. 13 days.	31.1	96	*36*	12	3.05
*a*17	Sh.Sk. and L. 13 days.	31.6	96	*44*	10	4.45
*a*21	Sh.Sk. and L. 13 days.	35.2	101	*26*	6	4.5
*a*22	Sh.Sk. and L. 17 days.	35.3	102	*47*	14	3.6
*a*23	Sh.Sk. and L. 20 days.	31.8	104	*72*	25	2.9
*a*24	Sh.Sk. and L. 189 days; killed February.	40.5	279	*39*	10	4.1
*a*25	Sh.Sk. and L. 197 days; killed April.	46.0	328	*36*	10	3.5
	Average			*42.3*	13.5	

All animals included in this table are the offspring of the same female.
For explanation and abbreviations see explanation to Table I.

included in this table are the offspring of the same female. The average number of follicles in this brood is 42, the smallest number was 24, the largest 72. Among 31 animals there were seven possessing more than 50 follicles, and only three whose thyroids contained more than 60 follicles.

In Table IV. are compared two series which were raised under the same conditions, but were the offspring of two different females. There is a marked difference in the follicular number between the two series. The average numbers show a difference of 15.

In Table V. "lot *a*" of the series CCLXXI. is compared with "lot *b*" of the same series. Of the thyroids of the 8 animals of "lot *b*" seven (87.5 per cent.) contain more than 40 follicles, three (37.5 per cent.) more than 65 follicles; in "lot *a*" the thyroids of only two animals (8.3 per cent.) among 24, contain more than 65 follicles, 15 (62.5 per cent.) more than 40 follicles. The average number of follicles in "lot *a*" is 44, in "lot *b*" 54. Both lots are the offspring of the same female. But in "lot *a*" the larvæ were isolated, at an early stage, into individual finger-bowls; individual attention was given to each animal both in regard to food and water. The larvæ of "lot *b*" were left together in one large dish; the supply of oxygen was poor and the food scarce. Only at the end of the larval period they were placed under conditions similar to those of "lot *a*." The follicular number of the animals raised under favorable conditions is lower than the follicular number of those raised under poor conditions.

In Table VI. "lot *b*" of the preceding series is compared with series CCLXII. As mentioned before, "lot *b*" consisted of animals raised under unfavorable conditions of food and oxygen. Series CCLXII. was composed of larvæ of unknown parentage, collected in one of the ponds of the vicinity of Woods Hole and brought to the laboratory only shortly before metamorphosis These animals were extremely well nourished, of large size and had lived under optimum conditions. The difference in the follicular number between this series and "lot *b*" of the series CCLXXI. is still greater than the difference between the well nourished "lot *a*" of series CLXXI. and the "lot *b*." In series

Table IV.

The Progeny of Two Different Females of *Ambystoma maculatum*, Raised under the Same Conditions, are Compared Regarding the Number of Follicles of the Thyroid Gland.

Series CCLXXIV.						Series CCLXXI.*b*.					
Number.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm^2. Area.	Number of Follicles per cm^2. Area.	*Number of Follicles.*	Age in Days.	Body Length in mm.	Stage of Animal.	Number.
1	Larval.	20.0	65	*52*	43						
2	Larval.	21.3	66	*28*	22						
*a*1	E.pr.d.	29.2	92	*49*	14						
*a*2	E.pr.d.	32.3	92	*29*	7	14	*41*	79	27.2	E.pr.sl.; yell.N.	2
						17	*56*	87	30.6	E.pr.d.; yell.N.; Op.shall.	3
*a*3	Pieces 1 day; E.pr.d.; yell.N.	33.7	96	*32*	11	11	*72*	88	31.1	E.pr.d.; Op.shall.	5
						14	*47*	91	32.4	Just shedding; Op. and G. open.	6
						17	*43*	77	27.1	Just Sh.Sk.; Op. and G. closed.	1
3	Sh.Sk. 0 days.	22.5	68	*48*	19						
*a*5	Sh.Sk. and L. 1 day.	—	106	*26*	10						
						16	*71*	92	31.7	Sh.Sk. and L. 2 days.	7
						21	*67*	95	28.6	Sh.Sk. and L. 3 days.	8
						17	*32*	87	28.8	Sh.Sk. and L. 4 days.	4
*a*4	Sh.Sk. and L. 5 days.	35.4	100	*47*	16						
	Average			*39*	18	16	*54*				

Table V.

Two Series of *Ambystoma maculatum*, Composed of the Offspring of the Same Female, but Raised under Different Conditions, are Compared with Each Other Regarding the Number of Follicles of the Thyroid Gland.

Series CCLXXI.*a*.						Series CCLXXI.*b*.					
Number.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm.² Area.	Number of Follicles per cm.² Area.	*Number of Follicles.*	Age in Days.	Body Length in mm.	Stage of Animal.	Number.
1	E.pr.sl.	25.7	75	*42*	17						
2	E.pr.sl.	27.9	77	*51*	16						
4	E.pr.d.	31.1	77	*35*	15	14	*41*	79	27.2	E.pr.sl.; yell.N.	2
						17	*56*	87	30.6	E.pr.d.; yell.N.	3
5	E.pr.c.; yell.N.	35.9	88	*35*	13	11	*72*	88	31.1	E.pr.d.; yell.N.	5
6	E.pr.c.; Op. and G. closed.	35.6	89	*65*	12						
3	Just shedding; Op. and G. closed.	27.8	77	*30*	10	14	*47*	91	32.4	Just shedding; Op. and G. open.	6
12	Just Sh.Sk.; Op. and G. open.	—	91	*28*	9	17	*43*	77	27.1	Just Sh.Sk.; Op. and G. closed.	1
14	Sh.Sk. 0 days; Op. and G. open.	35.2	95	*24*	8						
7	Sh.Sk. 1 day; Op. and G. closed.	33.8	89	*50*	12						
9	Sh.Sk. and L. 2 days; Op. and G. closed.	31.6	89	*52*	11						
8	Sh.Sk. and L. 2 days; Op. and G. closed.	33.5	89	*42*	8	16	*71*	92	31.7	Sh.Sk. and L. 2 days; Op. and G. closed.	7
13	Sh.Sk. and L. 2 days; Op. and G. closed.	34.8	92	*44*	6						
10	Sh.Sk. and L. 3 days; Op. and G. closed.	32.2	89	*57*	13	21	*67*	95	28.6	Sh.Sk. and L. 3 days; Op. and G. closed.	8
11	Sh.Sk. and L. 4 days.	29.6	89	*43*	10	17	*32*	87	28.8	Sh.Sk. and L. 4 days.	4
15	Sh.Sk. and L. 6 days.	33.1	96	*42*	7						
18	Sh.Sk. and L. 11 days.	32.2	100	*63*	15						
19	Sh.Sk. and L. 11 days.	33.1	101	*36*	15						
20	Sh.Sk. and L. 12 days.	34.9	101	*50*	9						
16	Sh.Sk. and L. 13 days.	31.1	96	*36*	12						
17	Sh.Sk. and L. 13 days.	31.6	96	*44*	10						
21	Sh.Sk. and L. 13 days.	35.2	101	*26*	6						
22	Sh.Sk. and L. 17 days.	35.3	102	*47*	14						
23	Sh.Sk. and L. 20 days.	31.8	104	*72*	25						
	Average			*44*	12	16	*54*				

TABLE VI.

TWO SERIES OF *Ambystoma maculatum*, ONE RAISED UNDER OPTIMUM CONDITIONS (CCLXII.), THE OTHER ONE UNDER POOR CONDITIONS (CCLXXI.*b*), ARE COMPARED WITH EACH OTHER REGARDING THE NUMBER OF FOLLICLES OF THE THYROID GLAND.

Series CCLXII.						Series CCLXXI.*b*.					
Number.	Stage of Animal.	Body Length in mm.	Age in Days.	*Number of Follicles.*	Number of Follicles per cm^2. Area.	Number of Follicles per cm^2. Area.	*Number af Follicles.*	Age in Days.	Body Length in mm.	Stage of Animal.	Number.
6	Larval; yell.N.	36.0	125	*10*	3	14	*41*	79	27.2	Larval; E.pr.sl.; yell.N.	2
4	Late larva; E.pr.	37.3	125	*35*	7	17	*56*	87	30.6	E.pr.d.; yell.N.; Op.shall.	3
10	Late larva; E.pr.	37.4	127	*30*	7	11	*72*	88	31.1	E.pr.d.; yell.N.; Op.shall.	5
8	Begins Shedd.Sk.; Op.shall.	37.3	126	*37*	8						
7	Begins Shedd.Sk.; Op.shall.	42.0	126	*25*	3						
						14	*47*	91	32.4	Just shedding; Op. and G. open.	6
2	Just Sh.Sk.; Op. clos.; G. open.	42.4	126	*31*	4	17	*43*	77	27.1	Just Sh.Sk.; Op. and G. closed.	1
5	Sh.Sk. ½ hour; Op. and G. open.	40.1	125	*5*	1						
9	Sh.Sk. 3 hours; Op. very shall.	37.0	126	*44*	8						
11	Sh.Sk. and L. 1 day; Op. and G. clos.	36.1	128	*30*	7						
12	Sh.Sk. and L. 1 day; Op. and G. clos.	32.4	128	*37*	10						
1	Sh.Sk. 2 days; L. 1 day; Op. and G. clos.	40.9	120	*39*	5	16	*71*	92	31.7	Sh.Sk. and L. 2 days; Op. and G. clcs.	7
						21	*67*	95	28.6	Sh.Sk. and L. 3 days; Op. and G. clos.	8
14	Sh.Sk. 4 days; L. 3 days; Op. and G. clos.	41.4	130	*36*	4	17	*32*	87	28.8	Sh.Sk. and L. 4 days; Op. and G. clos.	4
	Average			*30*	6	16	*54*				

CCLXII. the average follicular number is only 30, while in "lot *b*" it is 54; there is a difference of 24 between the average follicular numbers of these two series. And it is again the series reared under better conditions, in which the follicular number is lower.

As far as we can trust these scanty figures it seems that the follicular number of the thyroid gland is influenced by hereditary and by environmental factors. Among the descendants of one particular female there may be more animals with conspicuously low or high numbers of follicles than among the descendants of other females. Animals growing up in a favorable environment have a lower number of follicles than those growing up in a less favorable environment.

We do not know what rôle the number of follicles might play in the function of the thyroid gland. Nevertheless it is known that certain pathological conditions are characterized by high or low numbers of follicles. It is of interest in this connection that the inclination towards a higher or lower number of follicles may be inherited.

Summary.

1. In the species *Ambystoma maculatum*, as contrasted to the species *A. opacum*, the number of follicles does not show, as a rule, the characteristic changes related to metamorphosis and consisting in the formation, by fusion of follicles, of a large, tube-like or bag-like follicle filled with colloid. Nevertheless a large, bag-like follicle making the largest part of the entire organ was found in one animal among 120.

2. In the species *A. maculatum* the average number of follicles is 39.7.

3. The number of follicles in each thyroid remains constant during the entire life of the individual.

4. The growth of the thyroid is effected entirely by an increase in the size of the follicles.

5. The primary follicles are simple spherical vesicles.

6. As the follicles grow in size the surface becomes markedly irregular. The large follicles possess diverticula of varying number, size and shape or may be composed of two or more equally large and diverticulated component follicles.

7. Separation of the bud-like diverticula from the mother follicles or cleavage of the larger follicles into smaller follicles does not occur.

8. The bud-like diverticula and composite follicles are the result of fusion between adjacent follicles.

9. The source for the continuous formation of new follicles are probably small masses of epithelial cells found in many thyroids.

10. Owing to the fusion of the primary follicles, which goes on at the same rate as the formation of new follicles, the number of follicles remains constant.

11. In adult, sex-mature animals primary follicles cease to develop; those present do not fuse with larger follicles, but themselves begin to enlarge in size.

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